

# Plastic responses to temporal variation in moisture availability: consequences for water use efficiency and plant performance

Joshua J. Picotte · David M. Rosenthal ·  
Jennifer M. Rhode · Mitchell B. Cruzan

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**Abstract** The ability to appropriately modify physiological and morphological traits in response to temporal variation should increase fitness. We used recombinant hybrid plants generated by crossing taxa in the *Piriqueta caroliniana* complex to assess the effects of individual leaf traits and trait plasticities on growth in a temporally variable environment. Recombinant hybrids were used to provide a wide range of trait expression and to allow an assessment of the independent effects of individual traits across a range of genetic backgrounds. Hybrid genotypes were replicated through vegetative propagation and planted in common gardens at Archbold Biological Station in Venus, Florida, where they were monitored for growth, leaf morphological characters, and integrated water use efficiency (WUE) (C isotope ratio;  $\delta^{13}\text{C}$ ) for two successive seasons. Under wet conditions only leaf area had significant effects on plant growth, but as conditions became drier, growth rates were greatest in plants with narrow leaves and higher trichome densities. Plants with higher WUE exhibited increased growth during the dry season but not during the wet season. WUE during the dry season was increased for plants with smaller, narrower leaves that had higher trichome densities and increased reflectance. Examination of alternative path models revealed that during the dry season leaf traits had significant effects on plant growth only through their direct

effects on WUE, as estimated from  $\delta^{13}\text{C}$ . Over the entire growing season, plants with a greater ability to produce smaller and narrower leaves with higher trichome densities in response to reduced water availability had the greatest growth rate. These findings suggest that plants making appropriate changes to leaf morphology as conditions became dry had increased WUE, and that the ability to adjust leaf phenotypes in response to environmental variation is a mechanism by which plants increase fitness.

**Keywords** Adaptive plasticity · Carbon isotope ratio · Drought · Hybrid · *Piriqueta*

## Introduction

Organisms that experience changing environments often respond by making physiological and morphological modifications that affect their survival and reproduction. While phenotypic responses to temporal environmental variation are probably common and may be important for adaptation to variable environments (Alpert and Simms 2002; Gomulkiewicz and Kirkpatrick 1992; Levins 1968; Schlichting and Pigliucci 1995), research on phenotypic plasticity has primarily focused on spatial variation (reviewed in Pigliucci 2001), and relatively few studies examine phenotypic responses of individuals to changing environments (Martinez-Garza and Howe 2005; Winn 1996a, b). Moreover, it has been difficult to demonstrate the adaptive value of phenotypic plasticity in response to fine-grained temporal variation (i.e., environmental variation experienced by individuals within a single growth cycle: Miner and Vonesh 2004; Winn 1999). Considering the fitness consequences of environmental variation within a single season is particularly appropriate for perennial plants since their sedentary

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J. J. Picotte · D. M. Rosenthal · M. B. Cruzan (✉)  
Department of Biology, Portland State University,  
PO Box 751, Portland, OR 97207, USA  
e-mail: Cruzan@pdx.edu

J. M. Rhode  
Department of Biology, University of North  
Carolina at Asheville, Asheville, NC 28804, USA

nature precludes many avoidance responses (i.e., other than dormancy: Harper 1977), and their modular organization enables phenotypic adjustments in “real time” as new modules develop under changing conditions (Chu et al. 2006; de Kroon et al. 2005; Huber et al. 1999; Sultan 2005; Watson and Casper 1984; White 1979).

Of the types of environmental variation that plants typically experience, changes in levels of moisture and temperature are probably the most critical to their survival and reproduction (Lambers et al. 1998; Nobel 2005). This is especially true for plants growing in seasonally arid habitats, where extremes of water availability and temperature may be common (Fischer and Turner 1978; Schwinning et al. 2004). Plants growing in these habitats may produce leaves that are smaller (Dudley 1996b) or have increased trichome densities (Ehleringer 1982) in response to increasing drought. We expect these modifications to be adaptive since smaller or narrower leaves reduce heat load through convective cooling (reviewed in Gates 1980; Gibson 1998; Givnish 1987; Nobel 2005). Similarly, higher trichome densities may reduce leaf temperatures by reflecting more incident sunlight (Ehleringer 1976; Ehleringer and Mooney 1978; Ehleringer and Werk 1986). Trichomes may also increase the leaf’s boundary layer (thin layer of stagnant air adjacent to the leaf surface), which will decrease the amount of water lost by evapotranspiration (Gates 1980; Schuepp 1993). Decreased leaf area and higher trichome densities may result in higher water use efficiency (WUE; defined as the total amount of uptake of CO<sub>2</sub> per amount of water loss), which is often characteristic of plants growing in xeric environments (Ehleringer and Cooper 1988; Hamerlynck et al. 2004; Rosenthal et al. 2005; Smith and Nobel 1977). Plants that are able to increase trichome density and reduce leaf surface area as plastic responses to decreased water availability should have higher fitness in arid environments (Ackerly et al. 2000). However, demonstrating that plastic responses of physiological traits are adaptive has been problematic (e.g., Caruso et al. 2006).

Our ability to delineate the ways in which individual traits and plastic responses influence fitness may be complicated by the fact that sets of characters may function in concert to confer adaptation to particular conditions. For example, adaptation to diff

this an ideal system for investigating phenotypic responses to drought and the adaptive value of individual leaf traits.

Here we measure leaf traits in third-generation recombinant hybrid genotypes and their plastic responses to temporal variation in water availability under field conditions to assess the influence of these traits on WUE, and its consequent effects on plant growth. Specifically, we examine the expression and plastic responses of a number of leaf traits including leaf area, shape (width/length ratio), trichome density, reflectance, and anthocyanin content during relatively wet and dry periods of the growth season. We estimated anthocyanin because hybrids vary in widely in their pigmentation (Picotte 2006) and anthocyanins protect the leaf by enhancing photo-chemical quenching of high-energy electrons that may damage cells and reduce the photosynthetic rate (Close et al. 2000; Mendez et al. 1999). We were particularly interested in the effects of leaf morphological traits on WUE, measured here with C isotope ratio ( $\delta^{13}\text{C}$ ). Under similar atmospheric conditions, greater  $\delta^{13}\text{C}$  is generally interpreted as higher integrated leaf-level WUE and frequently greater C gain for  $\text{C}_3$  plants (Farquhar et al. 1989), but see Donovan and Ehleringer (1994) for a notable exception. Our analyses indicate that several leaf traits had strong influences on WUE and plant growth under drought conditions, and genotypes that had the largest changes in their leaf morphologies in response to drying conditions had the greatest biomass accumulation over the growing season.

## Materials and methods

### Crosses

We used third-generation recombinant-hybrid genotypes in these experiments to obtain a wide range of phenotypic expression and to reduce correlations among separate traits. Genotypes were generated in the fall of 2003 by haphazardly crossing pairs of second-generation recombinant hybrid genotypes within six lineages based on reciprocal  $F_1$  crosses (i.e., CH, HC, CV, VC, VH, and HV: first letter indicates the maternal parent) among three parental types (the C and V morphotypes and their hybrid derivatives, H). Second-generation recombinant genotypes had previously been generated by haphazardly crossing backcross genotypes (first-generation recombinant hybrids) within the same lineages. The proportional contribution of the C, V, and H genotypes to third-generation recombinant hybrid genomes and the cytoplasmic genotype (assuming maternal inheritance) for each plant were inferred from known pedigrees. The recombinant hybrids selected for this experiment displayed variation in leaf morphology that overlaps the range of variation seen among parental morphotypes and hybrid derivatives.

### Propagation and establishment of the common garden

Fruits from crosses among second-generation recombinant hybrids were collected 15–18 days after hand pollination in the greenhouse, allowed to dry for at least 18 days, planted into 6.35 × 6.35-cm pots, and placed under misters with twice daily watering for approximately 2 months. Four to six cuttings were made from each mature recombinant hybrid plant by first dipping them into rooting hormone (Schultz TakeRoot) and then inserting them into Jiffy-7 peat pellets (Jiffy Products). The cuttings were kept under greenhouse conditions (watered 3 times daily for 10 min with an overhead water misting system, 27–35°C day/night temperature, and 10 h day length) for 1 month. A total of 900 cuttings representing 265 unique recombinant hybrid genotypes were planted in a common garden at the Archbold Biological Station (ABS) in Venus, Florida in late July 2004. The common garden was located on Red Hill in an area of Florida scrub that has deep yellow sand soil, which is characteristic of the long-leaf pine and turkey oak scrub habitats where C and H populations of *Piriqueta* often occur. Cuttings were planted in a randomized block design that consisted of 60 blocks of 15 plants each. The block design was included in the data analyses to account for microenvironmental variation.

### Plant growth measurements

Plants in the common garden were allowed to establish during the late summer and fall of 2004, and measurements began in the spring of 2005. During the 2005 growing season, each plant was measured for its stem length and leaf number, and the total number of stems, leaves, buds, flowers, and fruits were counted every 6 weeks from May to September, for a total of three measurements (initial size in mid May, the beginning of July, and at the end of August in 2005). Of34 TwOf34 mn5(c/F1 1.1(u)6399.580c)-8.1(h)-

### Leaf morphology

In mid July and late August 2005, one leaf was taken from the first or second node (newest growth) from each plant in the garden over a 1- or 2-day period. Leaves were placed

Models for the analysis of leaf traits (leaf area, length/width ratio, trichome density, anthocyanin content, and reflectance—all entered as covariates) on plant size included estimates of genomic composition (entered as a covariate for each parental type) and cytoplasmic genotype (categorical, fixed effect) to account for the effects of unmeasured phenotypic traits that differed among the parental taxa. These models included variables for the block (location within the transplant garden) as a categorical random effect, and initial plant size for each growth period (i.e., early May and early July size) as a covariate. Separate analyses were conducted for each growth period (May–June and July–August) using PROC GLM of SAS (SAS 2002). These models analyze the change in plant size over time since we control for initial plant size by including it as a covariate, so we refer to this response as “plant growth.” Plant size estimates were log-transformed and leaf traits were log- or square-root transformed when necessary to produce approximately normal distributions.

#### Path analyses

The subsample of 75 plants for which  $\delta^{13}\text{C}$  was measured was analyzed to estimate the effects of leaf area, width/length ratio, trichome density, leaf reflectance, anthocyanin content, and  $\delta^{13}\text{C}$  on plant size at the end of each growth period. Data were analyzed separately for the May–June and July–August seasons using AMOS v.6.0 (Arbuckle 2005) to estimate standardized regression coefficients and to create path models. Several path models were explored by modifying the paths so that individual variables: (1) only had direct effects on plant size, (2) had both direct and indirect (through their effects on  $\delta^{13}\text{C}$ ) effects on plant size, and (3) only had indirect effects on plant size through  $\delta^{13}\text{C}$ . Models were compared using the second-order Akaike information criterion ( $\text{AIC}_c$ ) for small samples (i.e., for  $n/k < 40$ , where  $n$  is the sample size and  $k$  is the number of parameters in the model; Burnham and Anderson 1998, p. 66) calculated with the  $\chi^2$  values from AMOS analyses of each model as described by Angilletta et al. (2006).

#### Plasticity analyses

The degree to which changes in the expression of leaf traits affected biomass accumulation over both wet and dry seasons was analyzed by including initial trait values and the magnitude of change for each leaf trait in analyses of covariance (ANCOVA). Plasticity of each trait for each plant ( $T_{\text{pi}}$ ) is defined here as the difference in expression between the second ( $T_2$ ) and first ( $T_1$ ) time period, standardized by expression in the first time period:  $T_{\text{pi}} = [(T_2 - T_1)/T_1]$ . ANCOVA models for the analysis of the effects of plasticity on plant size at the end of August included the

effects of garden position entered as a random factor, and initial plant size in May, all July leaf trait values, and their plasticities ( $T_{\text{pi}}$ ) entered as covariates.

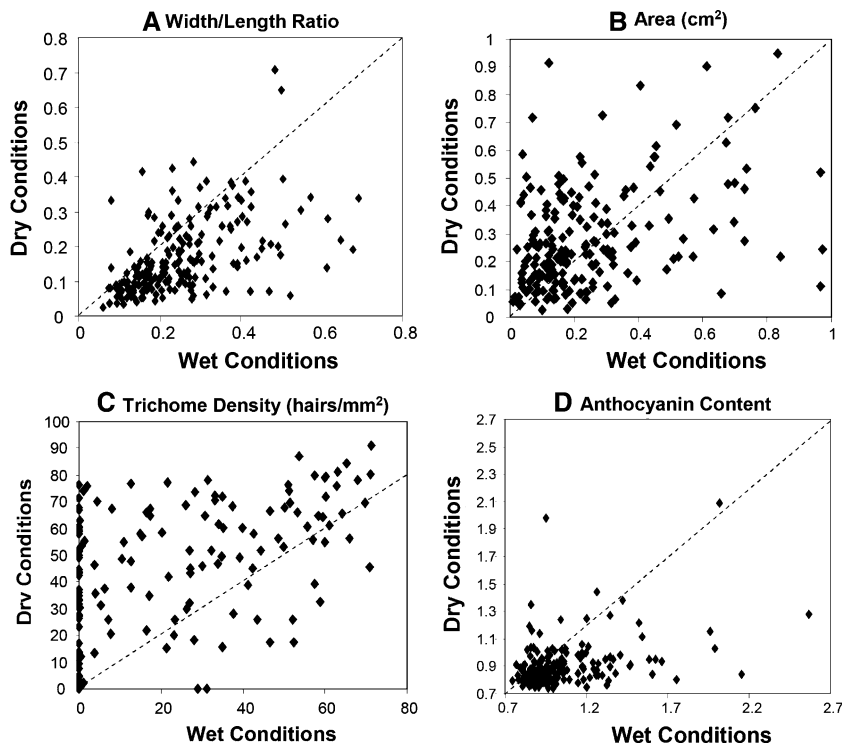
## Results

Leaf traits of plants in the transplant garden differed between the wet (May–June) and dry (July–August) growing seasons (Fig. 1). During the dry season of 2005 plants produced leaves with significantly lower width/length ratios ( $F = 98.56$ ,  $P < 0.0001$ ,  $1/406$  *df*), higher trichome densities ( $F = 46.73$ ,  $P < 0.0001$ ,  $1/406$  *df*), and lower anthocyanin content ( $F = 52.57$ ,  $P < 0.0001$ ,  $1/406$  *df*) than the same plants measured at the end of June (Fig. 1). While the leaves from the dry season were narrower (mean width = 0.267 vs. 0.235 cm for the wet and dry seasons, respectively;  $F = 13.92$ ,  $P < 0.0001$ ,  $1/406$  *df*), their overall surface area was larger ( $F = 248.68$ ,  $P < 0.0001$ ,  $1/406$  *df*) because they were significantly longer (mean length = 1.19 vs. 1.58 cm;  $F = 54.31$ ,  $P < 0.0001$ ,  $1/406$  *df*). Mean August leaf reflectance was almost identical to June reflectance (mean = 12.12% for both). Plants were nearly twice as tall at the end of August (mean height = 52.52) compared to June (mean height = 26.61;  $F = 55.43$ ,  $P < 0.0001$ ,  $1/325$  *df*), but the difference in the number of leaves was not as substantial (mean leaf number = 12.7 and 9.3 for August and June, respectively;  $F = 58.80$ ,  $P < 0.0001$ ,  $1/325$  *df*).

May plant size estimates were a good predictor of plant size at the end of the 2005 summer wet season (May–July; Table 1). Leaf area was the only trait that had significant effects on July plant size, so that plants with larger leaves had significantly greater growth during this wetter season. There were weaker effects of trichome density and leaf width/length ratio, where plants bearing wider leaves with fewer trichomes tended to have higher fitness, but these effects were not significant (Table 1). Block, cytoplasmic genotype, and the parental nuclear genomic composition parameters did not significantly affect July plant size in this model. When the covariates from this model were examined with path analysis the same trends were evident, and the overall path model explained 37% of variation in July plant size (path model not shown).

When a separate path analysis was conducted on a subsample of plants from the wet season for which we had determined WUE ( $\delta^{13}\text{C}$ ), the model with the lowest  $\text{AIC}_c$  value (indicating that it is the most plausible; Burnham and Anderson 1998) included direct effects of initial plant size, leaf area, and leaf width/length ratio with all other leaf characters acting indirectly through their effect on WUE ( $\delta^{13}\text{C}$  as estimated from single leaves collected from each plant at the end of each season; Fig. 2a). The  $\text{AIC}_c$  values for alternative models were substantially larger (i.e., all

**Fig. 1** Changes in leaf traits between the wet (May–July) and dry (July–August) seasons: leaf width/length ratio (a), total leaf area (b), trichome density (c), and anthocyanin content (based on reflectance estimates; d) for recombinant genotypes in the *Piriqueta caroliniana* complex. The dashed line in each panel represents no change in traits between the wet to the dry seasons. Values below the line indicate a shift to narrower leaves (a), smaller leaves (b), lower trichome densities (c), and lower anthocyanin content (d). For trichome densities, 50 genotypes did not have any leaf hairs in either season (points at the origin in c), and 52 genotypes shifted from no hairs in the wet season to varying trichome densities in the dry season (points along the y-axis of c)



**Table 1** ANOVA for the effects of garden position (Block), cytoplasmic genotype, percent parental genomic composition, leaf traits, and initial plant size on July 2005 size of recombinant hybrids in the *Piriqueta caroliniana* complex [morphotypes *caroliniana* (C) and *viridis* (V)]<sup>a</sup>

Source	df	Type III SS	Mean square	F-value	Pr > F
Block	11	9.87	0.90	1.55	0.1245
Cytoplasmic genotype	2	1.97	0.99	1.7	0.1871
Percent V	1	3.73	0.75	1.29	0.2746
Percent C	1	3.71	0.74	1.28	0.2767
Leaf area	1	4.50	4.50	7.77	0.0063
Leaf width/length	1	1.91	1.91	3.3	0.0721
Trichome density	1	2.06	2.06	3.56	0.0617
Reflectance	1	0.79	0.79	1.36	0.2455
Anthocyanin content	1	0.13	0.13	0.22	0.6372
Initial plant size	1	60.96	60.96	105.2	<0.0001

<sup>a</sup> The overall model was significant ( $F = 9.77$ ,  $P < 0.0001$ , 28/137 df)

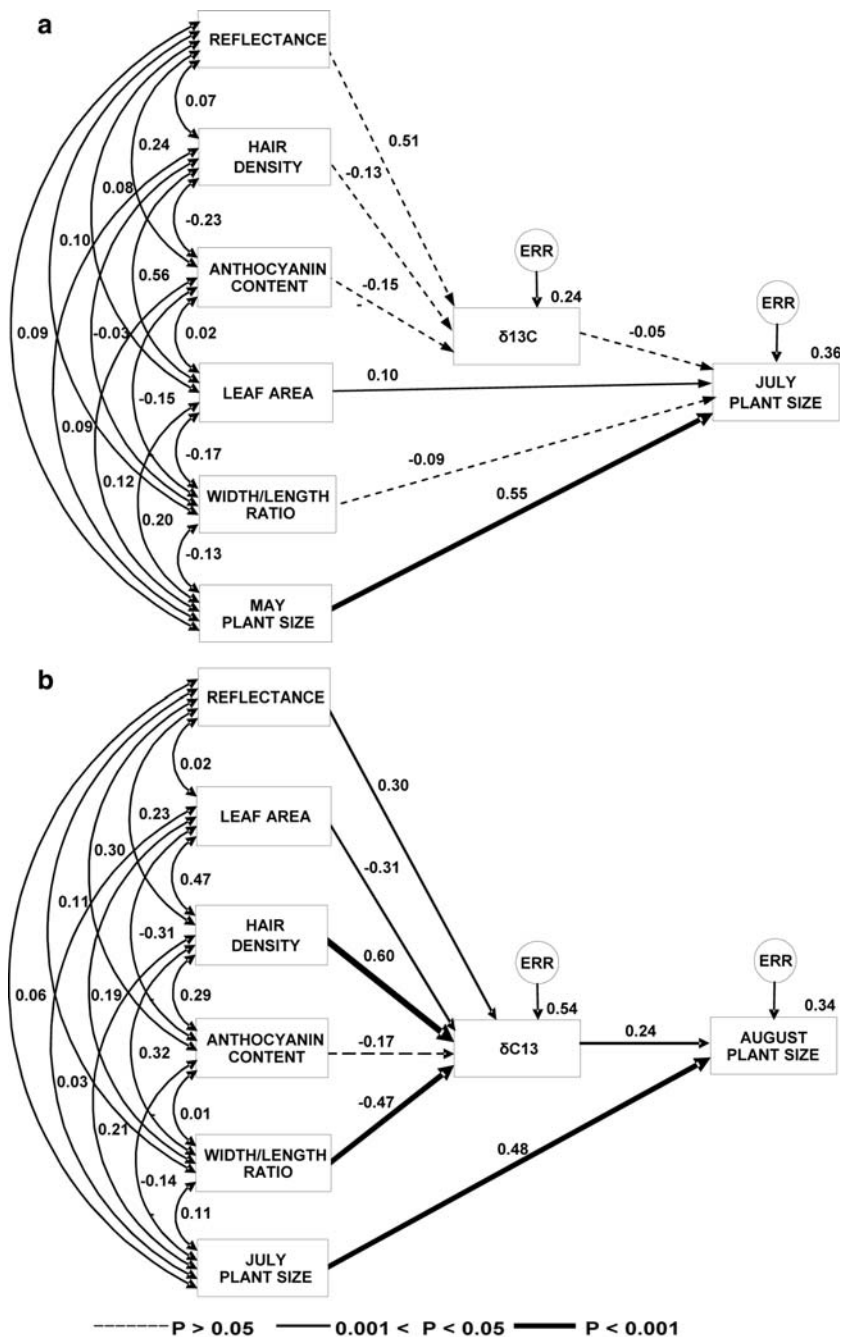
$\Delta_i > 2$ ) indicating that they did not warrant further consideration (Burnham and Anderson 1998, p. 70). The leaf characters explained 24% of the variation in WUE, but none of the individual leaf characters significantly influenced WUE measured from leaves collected at the end of the wet season (Fig. 2a). The results for this subsample are consistent with the full data set since initial plant size and leaf area had strong direct effects on the growth of plants during the wet period in both models (Table 1, Fig. 2a). Plants with high WUE appeared to have slightly slower growth during the wet season, but this relationship was not significant ( $P > 0.10$ ; Fig. 2a). The overall model explained 36% of the variation in growth for this subsample of plants (Fig. 2a).

Initial plant size was a good predictor of growth during the more arid period of the summer 2005 growing season

(July–August; Table 2). During this drier part of the summer plants that had narrower leaves and plants with higher trichome densities had significantly more growth. Block, cytoplasmic genotype, and parental genomic composition did not significantly affect plant size at the end of the drier growth period (Table 2). The path analysis using only covariates agreed with the results from the ANCOVA, where all the same covariates had significant direct effects on plant size (data not shown). Leaf trichome density, width/length ratio, and initial plant size significantly affected growth during the drier season, and the overall model for this time period explained 35% of the variation in plant size measured in August.

When levels of WUE for a subsample of plants from the dry season with  $\delta^{13}\text{C}$  estimates were analyzed in separate path models, results were largely consistent with those

**Fig. 2** Path analysis of the effects of wet (a) and dry (b) season leaf characters of recombinant hybrids in the *P. caroliniana* complex on plant growth through their effects on water use efficiency as inferred from C isotope ratios ( $\delta^{13}\text{C}$ ). The numbers above the right corner of the boxes for the response variables (July and August plant size and  $\delta^{13}\text{C}$ ) indicate the proportion of variance explained ( $R^2$ ) by each set of independent variables. The straight solid black or dashed arrows and their associated numbers represent the standardized regression coefficients (slope). The double arrows and their associated numbers represent positive or negative correlations between traits. *ERR* Error associated with  $\delta^{13}\text{C}$  and July or August plant size, respectively



from the full data set (Table 2, Fig. 2b). The path model with the lowest  $\text{AIC}_c$  value included only indirect effects of leaf characters on plant size through their effects on WUE, and the direct effect of initial plant size on plant size at the end of the season. The  $\text{AIC}_c$  values for alternative models indicated that they did not warrant further consideration (i.e., all  $\Delta_i > 2$ ). Levels of WUE were affected by leaf reflectance, leaf area, leaf trichome density, and leaf width/length ratio, with all the leaf characters combined explaining 54% of the variation in WUE. Both WUE and initial plant size had significant effects on plant size at the end of the dry season, and the overall model explained 34% of the

variation in plant size measured at the end of August (Fig. 2b).

The nuclear genetic composition of plants had significant effects on leaf characters and plant growth during the wet and dry seasons. Note that the contributions of all three parental morphotypes summed to one, making it impossible to assess all of them in the same model, so we chose to analyze only the proportion of C and V genomic contributions. The proportion of the nuclear genomic composition from the V parental morphotype significantly affected wet and dry season leaf area ( $F = 18.90$ ,  $P < 0.0001$ , slope =  $\downarrow 0.45$  and  $F = 22.64$ ,  $P < 0.0001$ , slope =  $\downarrow 0.51$ ), length/width

**Table 2** ANOVA for the effects of garden position (*Block*), cytoplasmic genotype, percent parental genomic composition, leaf traits, and initial plant size on August 2005 size of recombinant hybrids in the *P. caroliniana* complex<sup>a</sup>. For abbreviations, see Table 1

Source	<i>df</i>	Type III SS	Mean square	<i>F</i> -value	Pr > <i>F</i>
Block	11	13.77	1.38	1.86	0.0593
Cytoplasmic genotype	2	1.87	0.93	1.26	0.2879
Percent V	1	1.66	0.33	0.45	0.8151
Percent C	1	4.85	0.97	1.31	0.2658
Leaf area	1	1.68	1.68	2.26	0.1353
Leaf width/length	1	10.41	10.41	14.03	0.0003
Trichome density	1	4.21	4.21	5.68	0.0189
Reflectance	1	2.14	2.14	2.88	0.0924
Anthocyanin content	1	0.15	0.15	0.2	0.6562
Initial plant size	1	80.27	80.27	108.21	<0.0001

<sup>a</sup> The overall model was significant ( $F = 9.77$ ,  $P < 0.0001$ , 28/137 *df*)

ratio ( $F = 24.21$ ,  $P < 0.0001$ , slope =  $\downarrow$  0.38 and  $F = 73.43$ ,  $P < 0.0001$ , slope =  $\downarrow$  0.55), reflectance ( $F = 0.76$ ,  $P = 0.3841$ , slope =  $\downarrow$  0.23 and  $F = 9.17$ ,  $P = 0.0028$ , slope =  $\downarrow$  0.33), and trichome density ( $F = 24.58$ ,  $P < 0.0001$ , slope =  $\downarrow$  0.48 and  $F = 36.53$ ,  $P < 0.0001$ , slope =  $\downarrow$  0.61 for the wet and dry seasons, respectively; all slopes are standardized) while the effects on plant size ( $F = 4.62$ ,  $P < 0.0328$ , slope =  $\downarrow$  0.012 and  $F = 0.00$ ,  $P > 0.90$ , slope =  $\downarrow$  0.01) and anthocyanin content ( $F = 2.72$ ,  $P = 0.1004$  and  $F = 0.01$ ,  $P = 0.9050$ ) were weak. With the exceptions of anthocyanin content ( $F = 5.65$ ,  $P = 0.0185$ , slope =  $\downarrow$  0.34 and  $F = 8.23$ ,  $P = 0.0046$ , slope =  $\downarrow$  0.31) and leaf trichome density ( $F = 3.30$ ,  $P = 0.0707$ , slope =  $\downarrow$  0.07 and  $F = 6.54$ ,  $P = 0.0113$ , slope =  $\downarrow$  0.14), the effects of the proportion of the genome contributed from C parentals on leaf characters was much weaker ( $P > 0.15$  for all other characters). The cytoplasmic (maternal) genotype (C, V, or H) had a marginal effect on the dry season leaf width/length ratio ( $F = 4.85$ ,  $P < 0.089$ ; plants with a V cytotype had leaves that were narrower than plants with the C and H cytotypes), but not on any other leaf characters ( $P > 0.15$  for all). The only significant effect of genomic composition on plant growth was for the proportion of V genome in the wet season ( $F = 4.62$ ,  $P = 0.0328$ ) where higher proportions were associated with slower growth (standardized slope =  $\downarrow$  0.26).

Individual genotypes varied in their magnitudes and directions of changes in leaf traits (Fig. 1), and these plastic responses had significant effects on biomass accumulation over the wet and dry growth seasons (Table 3). Plants that had a larger decrease in leaf width/length ratio (slope =  $\downarrow$  29.17), increase in leaf trichome density (slope = 0.39), decrease in leaf area (slope =  $\downarrow$  11.79), and decrease in leaf anthocyanin content (a marginal effect, slope =  $\downarrow$  22.38; Table 3) between the wet and dry seasons were larger by the August growth measurement. In this same model the initial plant size in May (slope = 0.63), plant position in the garden (marginal effect), July leaf width/length ratio (slope =  $\downarrow$  4.67), and July trichome

density (a marginal effect, slope = 0.23) had affected final plant size at the end of the dry season. All other variables (genomic composition, initial leaf area, initial reflectance, initial anthocyanin content, and plasticity for reflectance) did not have significant effects on plant size at the end of the dry season (Table 3). With the exception of change in leaf width/length ratio between seasons [for maternal genotype,  $F = 3.61$ ,  $P = 0.0291$  (C > H = V), and for percent V,  $F = 5.80$ ,  $P = 0.0170$ ], these plasticity variables were not correlated with maternal genotype or nuclear genomic composition ( $P > 0.10$  for all).

## Discussion

Temporal variation in water availability to plants within the *Piriqueta caroliniana* complex has selected for the development of morphological and physiological adaptations that maximize the efficiency of water use relative to C acquisition. When subjected to temporal changes in water availability under natural conditions, these recombinant hybrid genotypes exhibited variation in vegetative growth that was dependent on each plant's leaf morphological traits and their ability to make appropriate morphological responses. Plants responded to changes in water availability by modifying leaf characters that affected WUE and biomass accumulation. Under wetter conditions, most plants had wider leaves and lower trichome densities. Under water stress conditions most of these recombinant genotypes modified their leaves to increase WUE by decreasing leaf width and increasing trichome density. The ability of these plants to acclimate to changes in moisture availability had a direct impact on their growth and reproductive effort. These results support the hypothesis that recombinant *Piriqueta* hybrids are responding to temporal variation in soil water availability by altering leaf traits that affect WUE. Moreover, it is apparent that these phenotypic changes are adaptive since plants that were able to make appropriate

**Table 3** ANOVA for the effects of leaf trait plasticity<sup>a</sup> on growth over the wet and dry seasons of recombinant hybrids in the *P. caroliniana* complex<sup>b</sup>. For abbreviations, see Table 1

Source	df	Type III SS	Mean square	F-value	Pr > F
Block	11	16.79	1.52	1.78	0.0672
Cytoplasmic genotype	2	0.34	0.17	0.20	0.8218
Percent V	1	0.39	0.39	0.46	0.4997
Percent C	1	0.10	0.10	0.11	0.7374
Leaf area	1	0.04	0.04	0.04	0.8348
Leaf width/length	1	14.22	14.22	16.60	<0.0001
Trichome density	1	2.70	2.70	3.16	0.0787
Reflectance	1	0.12	0.12	0.14	0.7072
Anthocyanin content	1	1.03	1.03	1.21	0.2745
Leaf area plasticity	1	5.07	5.07	5.92	0.0167
Leaf width/length plasticity	1	9.87	9.87	11.52	0.0010
Trichome density plasticity	1	6.94	6.94	8.11	0.0053
Reflectance plasticity	1	0.59	0.59	0.69	0.4070
Anthocyanin content plasticity	1	3.03	3.03	3.55	0.0625
Initial plant size	1	44.91	44.91	52.43	<0.0001

<sup>a</sup> Plasticity for each trait was calculated as the difference between the trait measured at the end of the wet ( $T_1$ : May–July) and dry ( $T_2$ : July–August) seasons of 2005 [ $(T_2 - T_1)/T_1$ ] for each individual plant

<sup>b</sup> The overall model was significant ( $F = 9.04$ ,  $P < 0.0001$ , 26/127 df)

morphological adjustments in response to changes in moisture availability had increased growth and reproductive effort over the entire growing season.

#### Leaf traits

We used recombinant hybrids in this experiment to provide a wider range of leaf morphological variation and to break up associations among traits. We expect that genotypes that occur in different habitats will differ for suites of morphological and physiological traits that may confer adaptation to their respective habitats. Non-recombinant genotypes would exhibit strongly associated combinations of morphological and physiological traits, making it more difficult to determine whether individual traits were affecting fitness. Although we used hybrids in this study, it is possible that the leaf traits we analyzed only appear to affect fitness because they are genetically correlated with other unmeasured traits (Lande and Arnold 1983). This is unlikely, however, because: (1) we did not find strong correlations among most of the traits that we measured, (2) we minimized the confounding effects of spurious correlations by measuring individual traits across a broad range of genetic backgrounds in recombinant hybrids, and (3) we controlled for the potential effects of unmeasured phenotypic traits by including measures of the genomic contributions of each of the parental taxa in our models. The traits that we measured were not strongly correlated with each other, but were often correlated with the genomic composition of hybrids, so it is

reasonable to expect that the residual effects of the variables estimating genomic contributions from the parental taxa reflect the contributions of unmeasured traits (e.g., stomatal density, osmotic potential of cells, cell wall elasticity, etc.) to variation in plant growth. While there is always the possibility that the apparent effects of traits measured in this study were due to association with unmeasured traits (e.g., due to cosegregation of alleles at quantitative trait loci due to linkage), the use of third-generation hybrids should provide sufficient opportunity for recombination to decompose the large majority of these associations.

In hot and dry environments increasing trichome density can be useful for reducing plant stress by reflecting radiant light to decrease leaf temperature (Ehleringer and Mooney 1978), and by increasing the boundary layer to reduce the rate of water loss to the environment (Nobel 2005). We found no correlations between leaf trichome density and reflectance, suggesting that leaf trichomes in the *P. caroliniana* complex do not reduce plant stress by reflecting light. However, both reflectance and trichome density had significant and positive effects on WUE, which led to increased plant fitness. It is likely that trichomes are increasing the leaf boundary layer, which would effectively decrease the amount of water loss from stomata and increase WUE (Parkhurst and Loucks 1972). The lack of an association between trichome density and reflectance, coupled with the lack of direct effects of leaf reflectance on plant growth, strongly suggests that leaf trichomes in *Piri-queta* function primarily to increase WUE by increasing the

thickness and stability of the leaf boundary layer rather than to reduce leaf temperature through reflectance of incident light.

A large proportion of these hybrid plants exhibited anthocyanin production in their leaves in July (90%) and August (70%). The production of anthocyanin in leaves was probably not beneficial, because anthocyanin had either a negative effect or no effect on plant size. This suggests that anthocyanin may be indicative of stress (Lee and Lowry 1980; Mendez et al. 1999) or cellular damage (Close and Beadle 2003) in the *P. caroliniana* complex and may not have a high production cost. However, it is also possible that the production of anthocyanin is reducing some other unknown physiological stress (reviewed in Gould 2004).

#### Water use efficiency

Higher (less negative) values of  $\delta^{13}\text{C}$  during the dry season (July–August) indicated that plants had higher WUE integrated over the life of the leaves sampled from each plant (Farquhar et al. 1989; Farquhar et al. 1982). This result agrees with previous studies that found that plants in drier sites have higher WUE (e.g., Dudley 1996a; Ehleringer and Cooper 1988; Rosenthal et al. 2005). Furthermore, plants with higher WUE had increased growth during the dry season, which is consistent with the results of Donovan and Ehleringer (1992), who found that WUE and plant size were positively correlated in a semiarid shrub. Although plants increased WUE during the dry season, this does not necessarily indicate that WUE always positively affects plant growth. We found that WUE during the wet season was negatively correlated with plant size, although this effect was not significant. This finding contrasts with results for the semiarid shrub *Chrysothamnus nauseosus* growing under well watered conditions, where more water use efficient plants were found to be larger (Donovan and Ehleringer 1994). The seasonal difference in WUE that we found in *Piriqueta* suggests that plants with better WUE are more tolerant of periods of drought. However, because the cost of higher WUE under more mesic conditions appears to be relatively low, plants with traits that decrease water loss may not have a substantial selective disadvantage during times of water abundance.

Previous studies have found that WUE can be significantly correlated with individual traits such as leaf area (Dudley 1996a). Our results agree with this, but more importantly, they suggest that multiple leaf traits (i.e., decreased leaf width, higher trichome density, and higher leaf reflectance) can interact to significantly increase WUE during drought and, to a lesser degree, negatively affect WUE when there is adequate soil moisture. Furthermore, the analysis of alternative path models indicated that the

effects of these leaf traits on plant size were indirect, and acted primarily through their effects on WUE, which in turn had a significant effect on plant growth under drought conditions.

#### Plasticity for leaf morphology

The importance of adaptive plasticity has been obscure because the majority of studies of phenotypic plasticity have not taken fitness into account (van Kleunen and Fischer 2005). However, it is hypothesized that if plants can respond appropriately to temporal changes in the environment, they may have a competitive advantage (Pigliucci 2001; Winn 1996a). The leaf morphology of hybrid plants in the *P. caroliniana* complex varied in response to temporal water availability, which indicates that these traits are plastic. However, not all genotypes responded to the decrease in water availability with the same degree or direction of change in trait expression, and some genotypes did not change trait expression at all (e.g., trichome density). Under drought conditions, plants that modified their leaves to be narrower and longer with higher trichome densities had increased growth, primarily through the effects of these leaf traits on WUE. This observed pattern of change in leaf morphology could be strictly a function of plant age (Martinez-Garza and Howe 2005; Winn 1996b); however, separate field and greenhouse experiments indicate that these plants are capable of decreasing trichome density and increasing leaf width in response to a change from low to high water availability (A. Henderson and M. B. Cruzan, unpublished data). Moreover, not all plants in the present study changed their leaf morphology in the same direction, and the plants that had the largest magnitude of change towards higher trichome densities and narrower leaves in response to decreased water availability had the highest vegetative growth over both the wet and dry seasons. This suggests that over time, plants that can make appropriate alterations to their leaf morphology in response to changing water regimes will be able to out-compete those plants that cannot respond appropriately.

If changes in leaf morphology increase the fitness of plants, then these plastic responses could be considered to be adaptive (Schlichting and Pigliucci 1995; Sultan 1987; Via and Lande 1985). Our data suggest that higher levels of leaf trait plasticity allow hybrid plants that are more flexible in the expression of leaf characters to have higher rates of growth under fluctuating environmental conditions. For example, the addition of leaf width/length plasticity, leaf trichome plasticity, leaf area plasticity, leaf reflectance plasticity, and leaf anthocyanin plasticity to the ANOVA models for growth over the entire 2005 season explained a significant proportion of the variance in plant size in August 2005. This result indicates that the degree to which

plants were able to change their leaf shape, size, trichome density, and amount of anthocyanin in response to changing environmental conditions contributed to biomass accumulation, which is consistent with the hypothesis that this response is an example of adaptive plasticity (Pigliucci 2001; Sultan 1987; van Kleunen and Fischer 2005).

Our results indicate that plants in the *P. caroliniana* complex exhibit adaptive plasticity in response to temporally variable environmental conditions. While much work has been conducted on plastic responses to spatially variable environments (reviewed in Pigliucci 2001), there is only one other published example of adaptive plasticity in response to temporal variation (Reboud and Bell 1997), and other studies have found temporal changes in morphology to be neutral or non-adaptive (e.g., Winn 1999). This is surprising, since most habitats exhibit some degree of temporal variation in environmental conditions and we would expect adaptive plasticity to be quite common. More importantly, this study suggests that multiple morphological traits can be both plastic and adaptive, and that suites of traits are responsible for adaptation to environmental conditions. These results also indicate that individual plants may respond to changing environments as they grow, and since their leaves are not able to change morphology once fully formed, an individual plant may possess a phenotypically diverse mosaic of leaves that developed under different conditions. Additional research on plasticity in response to temporal variation will continue to elucidate our understanding of the consequences of phenotypic flexibility for adaptation to variable environments.

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## References

- Ackerly DD, et al. (2000) The evolution of plant ecophysiological traits: recent advances and future directions. *Bioscience* 50:979–995
- Alpert P, Simms EL (2002) The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? *Evol Ecol* 16:285–297
- Angilletta MJ, Oufiero CE, Leache AD (2006) Direct and indirect effects of environmental temperature on the evolution of reproductive strategies: an information-theoretic approach. *Am Nat* 168:E123–E135
- Arbuckle JL (2005) AMOS SPSS, 6.0 edn. Amos Development, Spring House, Pa.
- Benz BR, Rhode JM, Cruzan MB (2007) Root anatomical and physiological responses to flooding in the *Piriqueta caroliniana* complex. *Am J Bot* 94:542–550
- Budyko MI (1974) *Climate and life*, 1st edn. Academic Press, New York
- Burnham KP, Anderson DR (1998) *Model selection and multimodal inference—a practical information-theoretic approach*, 2nd edn. Springer, New York
- Caruso CM, Maherali H, Sherrard M (2006) Plasticity of physiology in *Lobelia*: testing for adaptation and constraint. *Evolution* 60:980–990
- Chu Y, Yu FH, Dong M (2006) Clonal plasticity in response to reciprocal patchiness of light and nutrients in the stoloniferous herb *Glechoma longituba* L. *J Integr Plant Biol* 48:400–408
- Close DC, Beadle CL (2003) The ecophysiology of foliar anthocyanin. *Bot Rev* 69:149–161
- Close DC, Beadle CL, Brown PH, Holz GK (2000) Cold-induced photoinhibition affects establishment of *Eucalyptus nitens* (Deane and Maiden) Maiden and *Eucalyptus globulus* Labill. *Trees* 15:32–41
- Cruzan MB (2005) Patterns of introgression across an expanding hybrid zone: analyzing historical patterns of gene flow using non-equilibrium approaches. *New Phytol* 167:267–278
- Cruzan MB, Rhode JM (2004) Experimental analysis of adaptive landscape topographies. In: Cronk QCB, Whitton J, Ree RH, Taylor IEP (eds) *Plant adaptation: molecular genetics and ecology*. National Research Council of Canada, Vancouver, pp 61–69
- de Kroon H, Huber H, Stuefer JF, van Groenendael JM (2005) A modular concept of phenotypic plasticity in plants. *New Phytol* 166:73–82
- Donovan LA, Ehleringer JR (1992) Contrasting water-use patterns among size and life-history classes of a semiarid shrub. *Funct Ecol* 6:482–488
- Donovan LA, Ehleringer JR (1994) Potential for selection on plants for water-use efficiency as estimated by carbon-isotope discrimination. *Am J Bot* 81:927–935
- Dudley SA (1996) Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. *Evolution* 50:92–102
- Dudley SA (1996) The response to differing selection on plant physiological traits: evidence for local adaptation. *Evolution* 50:103–110
- Ehleringer J (1976) Leaf pubescence: effects on absorptance and photosynthesis in a desert shrub. *Science* 192:376–377
- Ehleringer J (1982) The influence of water stress and temperature on leaf pubescence development in *Encelia farinosa*. *Am J Bot* 69:670–675
- Ehleringer J, Cooper TA (1988) Correlations between carbon isotope ratio and microhabitat in desert plants. *Oecologia* 76:562–566
- Ehleringer J, Mooney HA (1978) Leaf pubescence: effects on absorptance and photosynthesis in a desert shrub. *Oecologia* 37:183–200
- Ehleringer J, Werk KS (1986) Modifications of solar-radiation absorption patterns and implications for carbon gain at the leaf level. In: Givnish TJ (ed) *On the economy of plant form and function*. Cambridge University Press, Cambridge, pp 57–81
- Farquhar GD, Ehleringer J, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annu Rev Plant Physiol Plant Mol Biol* 40:503–537
- Farquhar GD, O’Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust J Plant Physiol* 9:121–137
- Fischer RA, Turner NC (1978) Plant productivity in the arid and semi-arid zones. *Annu Rev Plant Physiol* 29:277–317
- Gates DM (1980) *Biophysical ecology*. Springer, New York
- Gibson AC (1998) Photosynthetic organs of desert plants. *Bioscience* 48:911–920
- Givnish TJ (1987) Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytol* 106:131–160

- Gomulkiewicz R, Kirkpatrick M (1992) Quantitative genetics and the evolution of reaction norms. *Evolution* 46:390–411
- Gould KS (2004) Nature's swiss army knife: the diverse protective roles of anthocyanins in leaves. *J Biomed Biotechnol* 5:314–320
- Hamerlynck EP, Huxman TE, McAuliffe JR, Smith SD (2004) Carbon isotope discrimination and foliar nutrient status *Larrea tridentata* (creosote bush) in contrasting Mojave Desert soils. *Oecologia* 138:210–215
- Harper JL (1977) Population biology of plants. Academic Press, London
- Heschel MS, Donohue K, Hausmann N, Schmitt J (2002) Population differentiation and natural selection for water-use efficiency in *Impatiens capensis* (Balsaminaceae). *Int J Plant Sci* 163:907–912
- Heschel MS, Riginos C (2005) Mechanisms of selection for drought stress tolerance and avoidance in *Impatiens capensis* (Balsaminaceae). *Am J Bot* 92:37–44
- Huber H, Lukacs S, Watson MA (1999) Spatial structure of stoloniferous herbs: an interplay between structural blue-print, ontogeny and phenotypic plasticity. *Plant Ecol* 141:107–115
- Lambers H, Lambers H, Pons T (1998) Plant physiological ecology, 1st edn. Springer, New York
- Lande R, Arnold S (1983) The measurement of selection on correlated characters. *Evolution* 37:1210–1226
- Lee DW, Lowry JB (1980) Young-leaf anthocyanin and solar ultraviolet. *Biotropica* 12:75–76
- Levins R (1968) Evolution in changing environments. Princeton University Press, Princeton, N.J.
- Lewis WH, Stripling HL, Ross RG (1962) Chromosome numbers for some angiosperms of the southeastern United States and Mexico. *Rhodora* 64:147–161
- Ludwig F et al (2004) Selection on leaf ecophysiological traits in a desert hybrid *Helianthus* species and early-generation hybrids. *Evolution* 58:2682–2692
- Malmstrom VH (1969) A new approach to the classification of climate. *J Geogr* 68:351–357
- Martin LJ, Cruzan MB (1999) Patterns of hybridization in the *Piriqueta caroliniana* complex in central Florida: evidence for an expanding hybrid zone. *Evolution* 53:1037–1049
- Martinez-Garza C, Howe HF (2005) Developmental strategy or immediate responses in leaf traits of tropical tree species? *Int J Plant Sci* 166:41–48
- Maskas SD, Cruzan MB (2000) Patterns of intraspecific diversification in the *Piriqueta caroliniana* complex in eastern North America and the Bahamas. *Evolution* 54:815–827
- Mendez M, Jones DG, Manetas Y (1999) Enhanced UV-B radiation under field conditions increases anthocyanin and reduces the risk of photoinhibition but does not affect growth in the carnivorous plant *Pinguicula vulgaris*. *New Phytol* 144:275–282
- Miner BG, Vonesh JR (2004) Effects of fine grain environmental variability on morphological plasticity. *Ecol Lett* 7:794–801
- Nobel P (2005) Physiochemical and environmental Plant physiology, 3rd edn. Academic Press, San Diego, Calif.
- Parkhurst DF, Loucks OJ (1972) Optimal leaf size in relation to the environment. *J Ecol* 60:505–537
- Picotte J (2006) Leaf morphological responses to environmental change in the *Piriqueta caroliniana* complex. Department of Biology, Portland State University, Portland, Oreg.
- Pigliucci M (2001) Phenotypic plasticity: beyond nature and nurture. John Hopkins University Press, London
- Reboud X, Bell G (1997) Experimental evolution in *Chlamydomonas*. III. Evolution of a specialist and generalist types in environments that vary in space and time. *Heredity* 78:507–514
- Rhode JM, Cruzan MB (2005) Contributions of heterosis and epistasis to hybrid fitness. *Am Nat* 166:E124–E139
- Rosenthal DM, Ludwig F, Donovan LA (2005) Plant responses to an edaphic gradient across an active sand dune/desert boundary in the Great Basin Desert. *Int J Plant Sci* 166:247–255
- SAS (2002) SAS/STAT user's guide, version 6, 4th ed. SAS Institute, Cary, N.C.
- Schlichting CD, Pigliucci M (1995) Gene-regulation, quantitative genetics and the evolution of reaction norms. *Evol Ecol* 9:154–168
- Schuepp PH (1993) Tansley review no. 59 Leaf boundary-layers. *New Phytol* 125:477–507
- Schwinning S, Sala OE, Loik ME, Ehleringer JR (2004) Thresholds, memory, and seasonality: understanding pulse dynamics in arid/semi-arid ecosystems. *Oecologia* 141:191–193
- Sims DA, Gamon JA (2002) Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sens Environ* 81:337–354
- Smith WK, Nobel PS (1977) Influences of seasonal changes in leaf morphology on water-use efficiency for three desert broadleaf shrubs. *Ecology* 58:1033–1043
- Sultan SE (1987) Evolutionary implications of phenotypic plasticity in plants. *Evol Biol* 21:127–178
- Sultan SE (2005) An emerging focus on plant ecological development. *New Phytol* 166:1–5
- Thornthwaite CW (1948) An approach to toward a rational classification of climate. *Geogr Rev* 38:55–94
- van Kleunen M, Fischer M (2005) Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytol* 166:49–60
- Via S, Lande R (1985) Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* 39:505–522
- Watson MA, Casper BB (1984) Morphogenic constraints on patterns of carbon distribution in plants. *Annu Rev Ecol Syst* 15:233–258
- White J (1979) The plant as a metapopulation. *Annu Rev Ecol Syst* 10:109–145
- Winn AA (1996a) Adaptation to fine-grained environmental variation: an analysis of within-individual leaf variation in an annual plant. *Evolution* 50:1111–1118
- Winn AA (1996b) The contributions of programmed developmental change and phenotypic plasticity to within-individual variation in leaf traits in *Dicerandra linearifolia*. *J Evol Biol* 9:737–752
- Winn AA (1999) Is seasonal variation in leaf traits adaptive for the annual plant *Dicerandra linearifolia*? *J Evol Biol* 12:306–313